American Heart Association®

Hypertension

Manuscript Submission and Peer Review System

URL: http://hype-submit.aha-journals.org

Title: PREGNANCY AND LONG-TERM MATERNAL CARDIOVASCULAR HEALTH:

PROGRESS THROUGH HARMONIZATION OF RESEARCH COHORTS AND BIOBANKS

Manuscript number: HYPE201506357R1

Author(s): Anne Cathrine Staff, Oslo University Hospital, Ullevål

Christopher Redman, University of Oxford

David Williams, University College London

Paul Leeson, University of Oxford

Kjartan Moe, University of Oslo, Oslo, Norway

Baskaran Thilaganathan, University of London

Per Magnus, Norwegian Institute of Public Health

Eric Steegers, Erasmus MC

Eleni Tsigas, Preeclampsia Foundation

Roberta Ness, University of Texas School of Public Health

Leslie Myatt, Oregon Health and Science University

Lucilla Poston, King's College London

James Roberts, University of Pittsburgh

"Brief Review" for Hypertension

PREGNANCY AND LONG-TERM MATERNAL CARDIOVASCULAR HEALTH: PROGRESS THROUGH HARMONIZATION OF RESEARCH COHORTS AND BIOBANKS

Anne Cathrine Staff, Christopher W.G. Redman, David Williams, Paul Leeson, Kjartan Moe, Basky Thilaganathan, Per Magnus, Eric A.P. Steegers, Eleni Z. Tsigas, Roberta B. Ness, Leslie Myatt, Lucilla Poston, James M. Roberts, for the Global Pregnancy Collaboration (CoLab)

Affiliations: University of Oslo and Dept. of Obstetrics and Dept. of Gynaecology, Oslo University Hospital, Oslo, Norway (A.C.S and K.M.); Nuffield Dept. of Obstetrics and Gynaecology and University of Oxford, UK (C.R.); University College London, London, UK (D.W.); Radcliffe Dept. of Medicine, Oxford, UK (P.L.); Depts. of Obstetrics and Gynaecology, St. George's Hospital, London, UK (B.T.); Division of Epidemiology, Norwegian Institute of Public Health, Oslo, Norway (P.M.); Dept. of Obstetrics and Gynecology, Erasmus Medical Centre, Rotterdam, The Netherlands (E.A.P.S.); Preeclampsia Foundation, Melbourne, FL USA (E.Z.T.); University of Texas School of Public Health, Houston, TX USA (R.B.N.); University of Texas Health Science Center San Antonio, San Antonio, TX USA (L.M.); King's College London, London, UK (L.P.); Magee-Womens Institute, Department of Obstetrics Gynecology and Reproductive Sciences, Epidemiology and Clinical and Translational Research, University of Pittsburgh, Pittsburgh, USA (J.M.R.).

A.C.S., C.W.G.R., P.M., E.A.P.S., E.Z:T., L.M., L.P. and J.M.R. are members of the Global Pregnancy Collaboration (CoLab) consortium.

Corresponding author:
Anne Cathrine Staff, Dept. of Obstetrics and Dept. of Gynaecology, Oslo University Hospital, location Ullevål, Kirkeveien 166, Post Box 4956 Nydalen, 0424 Oslo, Norway.
Telephone: 0047 41 30 30 81
Fax: 0047 22 11 76 93
E-mail: uxnnaf@ous-hf.no (and a.c.staff@medisin.uio.no)

Short title*: Pregnancy and Long-term CVD Research Harmonization

Word count of manuscript: 5046 (text only, not including 109 references, 1 Table and 3 Supplemental Files)

Abstract: Not applicable according to the HYPERTENSION format for the present "Brief Review" manuscript format.

Key words: **a**therosclerosis, biobanks, cardiovascular disease, harmonization, hypertension

pregnancy, preeclampsia, placenta.

1. Background: Why we need to better understand the associations between pregnancy and future cardiovascular health

In 2011, the American Heart Association added preeclampsia, gestational diabetes and delivery of a growth-restricted child as pregnancy-related risk factors for cardiovascular disease (CVD).[1] This move was applauded by the obstetric research community which for some years had emphasized the importance of pregnancy as a "stress test" for detecting women at excessive risk for premature CVD.[2-4]

CVD is the leading cause of death for men and women in high income and most low to middle income countries.[5] Globally, coronary artery disease kills more women than men[6] although women develop CVD 10-15 years later than men. Women frequently present with unrecognized CVD symptoms and are twice as likely as men to die of a first acute myocardial infarction if less than 50 years old.[7] The preclinical stages of CVD are evident from a young age and are modifiable through control of classic risk factors (insulin resistance/diabetes mellitus, obesity, lack of exercise, tobacco smoking, hypertension and hyperlipidemia).[8,9] In this regard pregnancy is a window of opportunity for identifying those women with perinatal complications who may benefit from early risk detection and early CVD prevention.

In this paper we summarize the associations between pregnancy, placenta-related pregnancy complications, and future maternal cardiovascular disease. We present established as well as more novel hypotheses, which may explain these epidemiological associations. The interventions that potentially could reduce risks of future cardiovascular disease are enumerated. To facilitate progress we suggest methods of harmonizing study designs, long-term follow-ups of pregnancy cohorts and biobanks, and pooling of the world's data in ways that can enhance the power of current and future research.

2. Associations between pregnancy complications and future CVD risk

2.1 Preeclampsia and fetal growth restriction and future CVD risk

Preeclampsia is a pregnancy specific multisystem disorder defined by new-onset hypertension

and proteinuria after gestational week 20, or new onset preeclampsia-associated signs in the absence

of proteinuria.[8] Preeclampsia requires the presence of a placenta or residual placental components

(postpartum preeclampsia), but the relative contributions of maternal predisposing factors versus

placental factors to its pathophysiology are not well delineated.[9] Women with essential

hypertension, obesity, pregestational diabetes mellitus and renal disease are at elevated risk for

developing preeclampsia. Several large population based studies demonstrate that women who have

had preeclampsia are at increased risk for later CVD and premature death compared to women with

healthy pregnancies.[10-14] Women who have experienced either preeclampsia or fetal growth

restriction have a two-fold increased risk compared to pregnancies with a normal outcome. When a

woman has both preeclampsia and fetal growth restriction the likelihood of cardiovascular disease

may be as much as 8-fold higher.[15;16] Recurrent,[12;14;17] more severe, and early-onset preeclampsia,

as well as preeclampsia with concurrent neonatal morbidity increase the risk of later life

CVD,[14;16;18;19] much more than gestational hypertension (without proteinuria or other preeclampsia-

associated features) or late-onset preeclampsia.[13;20-22]

2.2 Prematurity, miscarriage and future CVD risk

Women with a history of preterm birth (<37weeks gestation), even without pregnancy-

induced hypertension[23] or preeclampsia,[10;11;24] are twice as likely to die from CVD compared to

women who delivered at term. Spontaneous preterm labor is caused by multiple pathologic

processes;[25] nonetheless, delivery of a preterm or small for gestational age infant overall,

independent of smoking and other risk factors, has been shown to increase the risk of CVD death

and hospitalization later in life.[26] Although less frequently investigated, recurrent miscarriages have

also been linked to future CVD[27] and to endothelial dysfunction.[28] Additionally, recurrent

pregnancy loss is associated with pregnancy complications such as placental abruption and

hypertensive pregnancy disorders, which are independently associated with markers of

cardiovascular dysfunction, at least in the short-term.[29;30]

2.3 Diabetes mellitus in pregnancy and future CVD risk

Women who develop gestational diabetes have a 70% higher risk for future CVD than those with no history of the disorder, mostly attributed to an increased risk for developing type 2 diabetes.[31] As many as half of women with a pregnancy complicated by gestational diabetes mellitus develop type 2 diabetes within 5 years,[32] and the diabetes risk is reported as seven-fold as compared to normoglycemic pregnancies.[33] Whether pregnancy *per se* exacerbates the increased risk for later life CVD associated with pre-existing diabetes is not known. Women with type 1 diabetes seem, however, to be more at risk for developing retinopathy and nephropathy later in life if they suffered from preeclampsia.[34] Chronic kidney disease is considered an independent CVD risk factor[35] and nephropathy may therefore add to the overall CVD risk after a preeclamptic pregnancy in women with pregestational diabetes.

3. Normal pregnancy and future risk for CVD

Several studies report an association between the number of a woman's pregnancies, even without adverse outcomes, and maternal CVD risk,[36-40] whereas others fail to find such an association.[41;42] High number of children does not elevate CVD risk in men.[43] In a large Swedish population based registry study, parity was independently associated with future maternal CVD in a J-shaped fashion (where 2 births represented the lowest risk) following adjustment for socioeconomic factors and pregnancy-related complications. The highest risk was amongst women with more than 5 births.[40] The same J-shaped trend between number of births and maternal cardiovascular mortality was found in a recent study from the Norwegian Birth Registry, but only in women with less than 10 years of education.[44] The number of offspring does not seem to increase the CVD risk for the male partners, after correcting for obesity and metabolic risks,[38] suggesting a pathophysiologic effect from pregnancy, but this finding requires replication. Nulliparity per se has been associated with increased CVD risk,[40] but also subfertile women who eventually conceive and have a child are at increased risk for CVD, even after adjusting for CVD risk factors and adverse pregnancy outcomes, suggesting shared risk factors for CVD and infertility.[45]

Women who deliver either large or small birthweight for gestational age (LGA and SGA) infants have been identified as being at increased risk for future CVD.[46] However study results are inconsistent,[46] and the association may be influenced by the population prevalence of gestational and pregestational diabetes mellitus as these conditions lead to LGA babies. The association of both LGA and SGA with preeclampsia[47] further confounds the birthweight and CVD relationship. Perhaps because placental weight and newborn weight are highly correlated, low placental weight also appears to increase maternal risk for future CVD.[48] The impact of breast-feeding on long-term maternal CVD seems also to be protective.[49]

4. Pregnancy: mechanistic associations to future CVD

During pregnancy the maternal cardiovascular system undergoes substantial physiological adaptive changes,[50] which may also differ according to fetal gender and pregnancy outcome.[51] Repetitive cardiac stress could underlie a report of an association between the number of live births with a small, but significant, increase in left ventricular mass and a small reduction in left ventricular ejection fraction from middle age.[52] In addition the metabolic consequences of uncomplicated pregnancies could be potentially atherogenic,[53] which could be exaggerated in those with pre-existing dyslipidemia, for example in obese women or diabetics.

4.1 Preeclampsia/ placental dysfunction and mechanisms for increased maternal CVD risk

The most widely held hypothesis to explain the link between preeclampsia and CVD focuses on common risk factors.[54] Preeclampsia and CVD may share common genetic risk factors,[55;56] although specific genetic origins of preeclampsia and placental dysfunction remain ill defined. Both preeclampsia[57] and atherosclerosis[58;59] arise from vascular inflammation with its associated endothelial dysfunction. Common risks include obesity, diabetes mellitus, insulin resistance and hyperglycemia, dyslipidemia (including hypertriglyceridemia and small, dense LDL-particles),[60-64] hypertension, a family history of CVD,[65] and the metabolic syndrome.[18;66] Paradoxically, cigarette

smoking, which augments the risk for atherosclerosis and CVD, reduces the risk for preeclampsia in women who smoke in middle and late pregnancy.[67] The latter may be mediated by a modulatory effect of carbon monoxide on placental production of angiogenic and antiangiogenic factors.[68] The "antiangiogenic factor" sFlt1 (soluble fms-like tyrosine kinase 1; reviewed below as an important biomarker for early-onset preeclampsia) is lower in smokers than non-smokers during pregnancy.[69]

An alternative hypothesis suggests that pregnancy in general, and preeclampsia (and other placental disorders) in particular, worsen preexisting, subclinical CVD risk factors or even induce *de novo* risk as reviewed above. A large Norwegian population based study, whilst proposing that pre-pregnancy risk factors are more important, [70-72] also showed that most CVD risk factors remained significantly higher after preeclampsia following adjustment for pre-pregnancy values. Possibly, the dyslipidemia of preeclampsia could accelerate progression toward clinical and more advanced atherosclerotic lesions and hypertension.[73;74]

It is possible that products of the dysfunctional placenta in preeclampsia could permanently compromise the maternal cardiovasculature.[74;75] These could include inflammatory molecules in general as well as factors that perturb maternal 'angiogenic balance': increased circulating sFlt1 and sEng (soluble endoglin), and reduced placental growth factor (PlGF) as well as unmeasurable low levels of free VEGF (vascular endothelial growth factor) during pregnancy.[76] Although sFlt1 falls rapidly after delivery, a modest dysregulation several months and years after a preeclamptic pregnancy has been described.[77,78;79] Increased angiotensin II sensitivity and sFlt-1 response to angiotensin II infusion in women with previous preeclampsia has been reported, supporting lasting dysfunctional angiogenic responses.[80] Interestingly, agonistic autoantibodies against the angiotensin II type 1 receptor are present in many preeclamptic pregnancies, and may also persist postpartum in some cases and seem to correlate with dysregulated angiogenic biomarkers,[81] suggesting another potential molecular link between pregnancy and future CVD that merits further research. Studies prior to conception are needed to determine whether these pregnancy and postpartum findings reflect a pre-existing profile and/or placental dysfunction. Of relevance, a precipitating role for

preeclampsia *per se* has been implicated in the study of the serum proteome of an experimental mouse model in which preeclampsia was induced by adenovirus delivery of sFlt1. At 6 months postpartum, increased expressions of proteins related to CVD were found in comparison to the postpartum profile of normally pregnant mice.[82]

Stem cells, either maternal mesenchymal stem cells or endothelial progenitor cells (EPC), offer intriguing potential as mediators of persistent cardiovascular dysregulation caused by a dysfunctional placenta. Circulating EPC are reportedly reduced in preeclampsia,[83] but pre-pregnancy studies of EPC are lacking. EPC, markers of endothelial health, are similarly reduced in patients with essential hypertension, in whom EPC senescence is accelerated. It is possible, although not established, that the extremely low free VEGF concentrations associated with any pregnancy, and possibly even lower in early-onset preeclampsia or fetal growth restriction, could reduce EPCs. Both VEGF and PlGF increase EPC recruitment, mobilization and survival outside of pregnancy.[84;85] A reduction in EPC in pregnancy, such as observed in preeclampsia, could potentially affect long-term endothelial function.

The influence of pregnancy on the maternal heart,[52] and effects of preeclamptic pregnancies in particular, have recently been strongly implicated in long-term cardiovascular risk.[86] 80% of women with preeclampsia show an "adaptive response" to the increased afterload of preeclampsia by left ventricular remodeling. One year postpartum, even in the absence of hypertension, one third of previously preeclamptic women presented global diastolic and regional longitudinal systolic dysfunction with septal bulging, indicative of myocardial damage, possibly as a consequence of ischemia or fibrosis. These changes were more severe and more frequent when associated with preterm, rather than term preeclampsia.[86] The long-term CVD outcome remains unknown, [75] but as diastolic dysfunction is recognized to predate heart failure and increased mortality,[87;88] poor long-term cardiovascular health is likely.

5. The important research questions

One of the most important questions is: does pregnancy cause or reveal an increased risk for CVD problems? The primary issues are of pre-pregnancy predisposition, the effect of pregnancy itself, and exaggeration of risk by pregnancy complications. These can only be resolved by new, and necessarily expensive prospective longitudinal cohort studies of women pre- and post- pregnancy. Medical management will be much better targeted and evidence-based once these issues have been clarified.

5.1 Optimal long-term medical supervision of women after pregnancies associated with increased CVD risk

In general, it is recommended that following pregnancy, women with pre-existing renal or cardiac complications or suffering from diabetes mellitus, should be offered appropriate specialist follow-up to assess CVD risk and reduce ultimate CVD morbidity. The advice for clinical follow-up of an otherwise healthy woman following complication in pregnancy associated with higher CVD risk is however fragmentary, and there is no global consensus (Supplemental Table 1). Furthermore many of the recommendations recognize the inadequacy of informative data,[89] and in the case of the American College of Obstetricians and Gynecologists (ACOG), the recommendations are presented only as suggestions.[8] For women with gestational diabetes mellitus (GDM), several guidelines (Supplemental Table 1) recommend routine oral glucose tolerance testing postpartum, or measurements of fasting glucose and HbA1c.[32] Adherence to these postpartum recommendations is generally unknown, and long-term follow-up recommendations after GDM are lacking in most guidelines. Currently there are no recommendations for maternal follow-up after premature delivery, fetal growth restriction, SGA or recurrent pregnancy loss in relation to future CVD.

A much better understanding of the natural history and time-course of progression towards cardiovascular disease after at-risk pregnancies is needed if evidence-based strategies for follow-up are to be more widely adopted. Demonstration of cost benefit is essential to convince policy makers and payers. We also need to know if early intervention would be more effective than current ad hoc

and unsystematic follow-up. An established risk score for CVD, the Framingham score, calculates the 10-year gender-specific risk for cardiovascular events. A young population is in general unlikely to suffer from CVD in the next 10 years. Framingham risk score is therefore very low for young women, even for those with classical and gender-independent risk factors for CVD such as diabetes and obesity.[90] This present risk score therefore seems inapplicable for young women, especially since the CVD risk associated with pregnancy disorders is not included. Indeed, the American Heart Association emphasizes that a low Framingham risk score is not sufficiently exclusive of risk for CVD in young women,[91] and have implemented lifestyle advice independent of this scoring system for women whose pregnancies were complicated by preeclampsia, fetal growth restriction, gestational diabetes or a premature delivery (Supplemental Table 1).

5.2 Pregnancy biomarkers and improved risk stratification for CVD and targeted intervention

Preeclampsia and preterm birth are associated with increased insulin resistance, dyslipidemia and inflammatory activation all relevant in the nonpregnant setting to cardiovascular disease. It is possible that the degree of abnormality could be relevant to later life CVD. Specific to pregnancy, maternal circulating angiogenic and antiangiogenic biomarkers are dysregulated in placenta-related pregnancy disorders.[77] Elevated circulating sFlt1 and low PlGF in pregnancy may have potential as predictors also of long-term CVD many years after pregnancy; a high sFlt1: PLGF ratio might direct postpartum interventions to those with greatest need. This hypothesis is readily testable in cohorts with postpartum clinical cardiovascular follow-up data.

Outside pregnancy, a high circulating PlGF (assumed to be endothelial-derived) is related to an increase in CVD events, but has only been investigated in elderly women with a previous CVD event.[92] There is little data on the associations between pregnancy and postpartum sFlt1and/or PlGF levels and not known if they play a role as potential biomarkers of future CVD risk. A continuing search for guidance of stratification by preeclampsia biomarkers is an important target for future research.

5.3 Therapeutic strategies to reduce long-term risk for CVD

Both the ACOG[93] and the UK NICE[94] guidelines include advice for women after pregnancy complications associated with increased CVD risk: to keep a healthy weight, engage in increased physical activity and refrain from smoking (Supplemental Table 1). The impact of short-term prolongation of a severely preeclamptic pregnancy, or of more aggressive antihypertensive therapy during pregnancy on the risk for future maternal CVD is uncertain. Also, the independent effect of a further pregnancy is not known: either if it is normal or complicated by recurrent preeclampsia. Since the early stages of atherosclerosis are reversible it is possible that prompt postpartum intervention (for example with statins, metformin, platelet inhibitors/anti-inflammatory drugs such as low dose aspirin, angiotensin-converting-enzyme inhibitors or angiotensin receptor blockers) could reduce CVD risk. But currently there is no evidence in favor of any intervention whether reserved for the highest risk groups or more widely applied.

6. Use of existing pregnancy cohorts and research biobanks

Collaboration between researchers who have existing pregnancy cohorts and biobanks across the world is a necessary prerequisite to solving the problems identified. By prolonging follow-up, using standardized protocols and combining data, it should be possible to establish if pregnancy or postpartum biomarker measurement can help stratify risk in seemingly healthy parous women whose pregnancy outcomes identify them as at risk for CVD. Angiogenic factors measured in pregnancy and postpartum[79] and related factors should be evaluated as CVD risk assessment tools, and "-omics" strategies could be employed to identify new candidate risk or pathophysiological factors. Such large datasets, with data collected in a unified way across multiple institutions and nations, could be a powerful guide to future intervention trials aimed at reducing the global burden of cardiovascular disease.

6.1 Potential pregnancy biobanks for long-term cardiovascular follow-up

The Global Pregnancy Collaboration (CoLab) (https://pre-empt.cfri.ca/colaboratory)

includes 30 international member centers, with data from more than 300 000 pregnancies and

biological materials from 20 000 pregnancies. The goal is to provide conclusive and globally

generalizable insight into disorders of pregnancy. The CoLab initiative has published

recommendations for standardizing clinical data and sample collection in studies of

preeclampsia.[95;96] It is also undertaking a pooling of individual measurements of placentally

associated biomarkers analyzed in 28 different cohorts worldwide.[97]

Several of the contributing pregnancy registries and biobanks within the CoLab network are

undertaking or planning long-term follow-up of maternal disease remote from pregnancy. Data and

samples have been usually acquired during pregnancy, and only rarely before pregnancy. The Dutch

Generation R study has followed a large population based cohort of women and their offspring after

pregnancy.[98-100] Another, the Norwegian MoBa study,[101;102] including more than 70,000 women and

100,000 pregnancies also has a long-term follow-up goal. One longitudinal UK study, OxWatch,[103]

is studying women from before pregnancy, during pregnancy and beyond. The Preeclampsia

Registry, developed and managed by the Preeclampsia Foundation and associated with CoLab, is

accepting participants worldwide, currently enrolling 2,000 women, most of whom have had

preeclampsia. The database also includes nulliparous and parous sisters, other family members, and

controls.

7. Harmonization of databases

The ability to merge the data or samples from different studies is limited by the heterogeneity in

how and which data are collected, as well as in the frequencies and intervals of clinical follow-up.

CoLab is developing an online clinical database, originally for prospective studies of preeclampsia,

which will standardize collection of appropriate data for pregnancy research and will be available

for general use in 2016. It follows up on previously recommended "minimal and optimal" datasets

for preeclampsia research,[95] and will facilitate pooling of data from such new prospective studies.

The principle of harmonization of study data can be extended to all other long-term health outcomes after pregnancy, uncomplicated or complicated. Any research study related to human pregnancy information (both within and outside the CoLab organization) is encouraged to register their pregnancy and/or long-term follow-up research study at an open web platform (www.linkregistry.org), to promote research collaboration across studies.

7.1 An ideal cohort to study remote CVD after pregnancies

An option, although costly, is to construct a new international, prospective, longitudinal, research cohort, which would commence prior to pregnancy, and follow women longitudinally over many years to include the "hard endpoints" of CVD (e.g. death, stroke, myocardial infarct). Such a cohort should be global in every sense. This "International Longitudinal Women's Health Cohort" will be challenging to fund and administer. Even without such a large formal cohort, we encourage that our recommendations of data storage harmonization are adopted for smaller individual studies to facilitate study linkages at a later date.

7.2 "Minimal" and "extended" follow-up research dataset for future CVD after pregnancy

Table 1 summarizes our suggestions for a "minimal" and "extended" research dataset for studying long-term cardiovascular health after pregnancy, including suggestions for "cardiovascular phenotyping", collection of general health assessments and pregnancy information. Recruitment should not be limited to women at elevated risk for CVD, but also uncomplicated pregnancies. Ideally, recruitment should be population based. The suggested follow-up in Table 1 focuses specifically on CVD, but could be modified to meet the needs of different health outcomes after pregnancy, such as renal, thyroid, neurodegenerative or psychiatric disease. Such studies could hopefully identify suitable time points for cost-efficient analyses of the follow-up of (apparently) healthy parous women after pregnancy complications.

In contrast, women in high income countries who have clinical evidence of CVD, either pre-pregnancy, during pregnancy or postpartum, would be followed up by a specialist (e.g., a

cardiologist), with clinical strategies that need individualization and may differ from the suggested research oriented suggestion of Table 1. Women with pre-pregnancy diabetes mellitus or renal disease should be offered appropriate specialist follow-up postpartum, whatever pregnancy complications, to reduce the risk for end-stage organ damage.

It is important that the data collected and the timing of collection in follow-up studies is similar across different studies (Table 1). We suggest that follow-up research studies after pregnancy obtain a clinical history, including data on smoking, hypertension and diabetes, obstetric history and length of breast feeding from all pregnancies,[95] and family history of CVD risk factors. In addition we suggest a "minimal" clinical assessment including blood pressure measurement, testing for insulin resistance (with fasting blood glucose as a first screening[1] or HbA1c or the more labor extensive oral glucose testing or HOMA (Homeostasis Model Assessment) score for the extended dataset). Our suggested testing for other risk factors for CVD, such as renal disease (urine dipstick for proteinuria as a first screening or albumin/creatinine ratio), BMI, total cholesterol, LDL-cholesterol, HDL-cholesterol etc., is consistent with CVD risk screening recommended by the American Heart Association.[1] Table 1 presents a "minimal dataset" and an "extended dataset" for research follow-up. The "minimal dataset" is chosen as information that can be collected also in low resource setting, in recognition of the necessity of information specific to settings with the highest rates of pregnancy complications and deaths from these conditions.

An "extended" follow-up research plan for CVD includes more detailed cardiovascular phenotyping as well as blood sampling for research purposes. Currently, there is no biomarker that is known to precisely predict future CVD in young and symptom-free women, who have normal kidney function, blood sugar, lipids and blood pressure, therefore adequate samples for various analytical options should be collected. Sampling and storage of biological material (blood, placenta, possibly urine and feces and other material) would cover a broad range of analytical options and biomarker discovery, including options for "-omics" (metabolomics, etc.). ^{1}H NMR metabonomics (a form of metabolomics related to nutrition) could for example explore atherosclerotic and CVD

pathophysiology.[104] Supplemental File 2 details the current most sensible options for extended cardiovascular phenotyping in a long-term follow-up clinical research setting, provided the necessary skill base is available. Linking imaging and physiologic phenotypes with later health outcomes, similar to approaches being used in large scale longitudinal cohorts such as UK Biobank,[105] may also identify novel vascular or cardiac risk markers that predict which women are at greatest risk for later cardiovascular disease.[91] Such studies typically use a comprehensive approach that captures data on a broad range of cardiovascular parameters, and often include assessment of other related systems through metabolic, bone, cerebral or renal imaging and assessment. We recognize that many pregnancy-associated research centers may have specialist experience or equipment for evaluation of only one, or a few, of these different areas but, through a collaborative approach, centers with similar data could be linked to generate combined datasets. In addition, there are some non-invasive techniques that do not require major infrastructure and so are widely available across multiple sites. Supplemental File 2 considers these common non-invasive techniques that could be incorporated into an extended follow-up dataset for research purpose and would allow comprehensive assessment across the woman's macrovasculature (both functional and structural investigations), microvasculature and heart.

7.2.1 Time points for measurement and frequency of postpartum research follow-up visits

The optimal time points for measurements of cardiovascular variables in longitudinal follow-up cohorts are unknown. Pre-pregnancy measures will be invaluable to discriminate those cardiovascular changes that predispose to pregnancy outcomes as opposed to those developing or aggravated by pregnancy itself. However, the challenges of a pre-pregnancy cohort include that women do not always plan their pregnancies, nor do pregnancies necessarily occur when planned. Thus the time between pre-pregnancy testing and the subsequent pregnancy will vary. Since vascular measures change with age, the gap between the time of measurement and the index pregnancy may reduce the value of the pre-pregnancy measure. It is not known whether or when in

pregnancy vascular phenotyping would be most relevant for unmasking the most reliable risk for long-term CVD. Until this question is resolved, testing could ideally be done in early, mid and late pregnancy, and, if possible, in the case of preeclampsia when the woman manifests the clinical signs.

The frequency of the suggested clinical research follow-up after pregnancy should be standardized to allow determination of the natural history of the progression to cardiovascular disease. Potential clinical findings at the follow-up will likely vary between premenopausal and postmenopausal women. Postmenopausal women have a higher short-time CVD risk, with greater disease prevalence. On the other hand, identifying increased risk in younger women would be optimal to prevent CVD, favoring more frequent and regular examinations of younger and clinically healthy women. For harmonizing purposes, we suggest a first 6-12 week postpartum follow-up after pregnancy, as this timing is used clinically today as a routine check-up after pregnancy in many countries. Thereafter, we suggest a 6 month and 1-year follow-up, with subsequent follow-up at least every 5 years for the clinically healthy women. If evidence of clinical CVD is found in this research setting, appropriate clinical and specialist follow-up is of course recommended. Importantly, even if the resources are not available for such work- intensive follow-up, we strongly recommend studies be designed to allow coupling of pregnancy data from the recruited women to other potential registries or patient databases documenting clinical (including "hard") CVD end-points.

Several countries currently offer population based health screening programs, such as screening for cervical and breast cancer, which are assumed to be cost-effective. However, CVD is the greatest cause of death and years of life lost in the world,[106] yet no screening is offered due to lack of evidence for its efficiency. In order to increase patient compliance and to secure a cost-efficient follow-up of CVD screening, our suggested "minimal" dataset follow-up could be adapted to such pre-existing screening programs, providing added value without much extra cost. This may

be a payer issue in countries without government-provided health care, which needs to be taken on by professional and consumer organizations as an advocacy initiative.

8.   The women's involvement in long-term follow-up for CVD

The involvement of women with relevant pregnancy experience in the development of these research programs is under-appreciated, but important and enlightening.[107] We therefore recommend that this research initiative is developed with continuing discussion with appropriate patient groups to ensure that their views are fully expressed and incorporated into research planning. Patient-run organizations such as, in the US (www.preeclampsia.org), UK (www.action-on-pre.eclampsia.org.uk) and Australia (www.aapec.org.au) actively support preeclampsia research and long-term follow-up for CVD. These organizations have a key role in updating women about the evidence for risks of CVD in relation to their pregnancy histories, even though women cannot expect routine follow-up without sound evidence that this is beneficial. But, women can strengthen the call for more and better research to gain the evidence. They can also help to update health personnel who are still relatively unfamiliar with the association of future CVD with pregnancy complications.[108] They can demand that hospitals provide more and better patient-oriented information regarding long-term CVD risk after pregnancy complications (e.g., www.themothersprogram.ca).

Supporting patient involvement should increase compliance with current (well-meant but often ignored) advice on weight control, smoking cessation, and management of additional CVD risk factors (e.g., diabetes and hyperlipidemia). The current US[93] and UK[109] advice on patient-oriented recommendations is very general. More CVD follow-up research is needed to enable better and more specific recommendations.

9.   Translation into clinical practice

We hope that in the future the longitudinal research studies, described here, will be translated into widely practiced, evidence-based routines of clinical follow-up, for selected women who have had

complicated pregnancies. A suggested template is given in Supplemental Figure 1. Resolution of the outstanding research issues that have been identified in this paper could identify which biomarkers would help to refine recommendations for, and timing of, the follow-ups and maximize health outcomes cost effectively. This or a similar template could be adapted to differing health systems and even linked to other established screening programs, for example those for cervical and breast cancer.

10. Perspectives

Despite a clearly documented increased risk for cardiovascular disease after pregnancy complicated by placental dysfunction or gestational diabetes, our understanding of the underlying mechanisms is poor. Nor is it clear how to appropriately target preventive strategies to the women at highest risk and what interventions are likely to confer benefit. More long-term research programs are needed particularly to discriminate between the specific effects of pregnancy and pre-pregnancy risk factors on future maternal CVD.

The need for adequately powered, large, longitudinal studies is identified as a critical issue. These are expensive and difficult to achieve in isolation. Progress will be faster if data and samples are collected in such a way that separate studies can be combined to achieve collaboratively determined goals that are otherwise unattainable. This would be powerfully facilitated by pre-agreed harmonization of research protocols to ensure that important data and samples can be readily pooled. We suggest a provisional format for such harmonization and encourage discussion, between those involved, to refine its design. In addition, to address the crucial question of the role of pre-pregnancy risk factors we promote the concept of a new "International Longitudinal Women's Health Cohort". It should then become possible to validate markers of long-term CVD in young women and identify new therapeutic targets for intervention, in collaboration with clinical experts on CVD. Better surrogate markers, singly or in combination, for long-term CVD in young women will enable targeted testing of primary prophylactic agents many decades prior to the first, and possibly lethal, evidence of atherosclerosis and cardiovascular disease.

Reference List

(1) Mosca L, Benjamin EJ, Berra K, Bezanson JL, Dolor RJ, Lloyd-Jones DM, Newby LK, Pina IL, Roger VL, Shaw LJ, Zhao D, Beckie TM, Bushnell C, D'Armiento J, Kris-Etherton PM, Fang J, Ganiats TG, Gomes AS, Gracia CR, Haan CK, Jackson EA, Judelson DR, Kelepouris E, Lavie CJ, Moore A, Nussmeier NA, Ofili E, Oparil S, Ouyang P, Pinn VW, Sherif K, Smith SC, Jr., Sopko G, Chandra-Strobos N, Urbina EM, Vaccarino V, Wenger NK. Effectiveness-based guidelines for the prevention of cardiovascular disease in women--2011 update: a guideline from the american heart association. *Circulation.* 2011;123:1243-1262.

(2) Sattar N, Greer IA. Pregnancy complications and maternal cardiovascular risk: opportunities for intervention and screening? *BMJ.* 2002;325:157-160.

(3) Rich-Edwards JW. Reproductive health as a sentinel of chronic disease in women. *Womens Health (Lond Engl).* 2009;5:101-105.

(4) Roberts JM, Hubel CA. Pregnancy: a screening test for later life cardiovascular disease. *Womens Health Issues.* 2010;20:304-307.

(5) Mosca L, Benjamin EJ, Berra K, Bezanson JL, Dolor RJ, Lloyd-Jones DM, Newby LK, Pina IL, Roger VL, Shaw LJ, Zhao D, Beckie TM, Bushnell C, D'Armiento J, Kris-Etherton PM, Fang J, Ganiats TG, Gomes AS, Gracia CR, Haan CK, Jackson EA, Judelson DR, Kelepouris E, Lavie CJ, Moore A, Nussmeier NA, Ofili E, Oparil S, Ouyang P, Pinn VW, Sherif K, Smith SC, Jr., Sopko G, Chandra-Strobos N, Urbina EM, Vaccarino V, Wenger NK. Effectiveness-based guidelines for the prevention of cardiovascular disease in women--2011 update: a guideline from the American Heart Association. *J Am Coll Cardiol.* 2011;57:1404-1423.

(6) Lawton JS. Sex and gender differences in coronary artery disease. *Semin Thorac Cardiovasc Surg.* 2011;23:126-130.

(7) Vaccarino V, Parsons L, Every NR, Barron HV, Krumholz HM. Sex-based differences in early mortality after myocardial infarction. National Registry of Myocardial Infarction 2 Participants. *N Engl J Med.* 1999;341:217-225.

(8) Report of the American College of Obstetricians and Gynecologists' Task Force on Hypertension in Pregnancy. Hypertension in pregnancy. *Obstet Gynecol.* 2013;122:1122-1131.

(9) Redman CW, Sargent IL, Staff AC. IFPA Senior Award Lecture: Making sense of pre-eclampsia - Two placental causes of preeclampsia? *Placenta.* 2014;35 Suppl:S20-S25.

(10) Irgens HU, Reisaeter L, Irgens LM, Lie RT. Long term mortality of mothers and fathers after pre-eclampsia: population based cohort study. *BMJ.* 2001;323:1213-1217.

(11) Smith GC, Pell JP, Walsh D. Pregnancy complications and maternal risk of ischaemic heart disease: a retrospective cohort study of 129,290 births. *Lancet.* 2001;357:2002-2006.

(12) Lykke JA, Langhoff-Roos J, Sibai BM, Funai EF, Triche EW, Paidas MJ. Hypertensive pregnancy disorders and subsequent cardiovascular morbidity and type 2 diabetes mellitus in the mother. *Hypertension.* 2009;53:944-951.

(13) Bellamy L, Casas JP, Hingorani AD, Williams DJ. Pre-eclampsia and risk of cardiovascular disease and cancer in later life: systematic review and meta-analysis. *BMJ.* 2007;335:974.

(14) Wikstrom AK, Haglund B, Olovsson M, Lindeberg SN. The risk of maternal ischaemic heart disease after gestational hypertensive disease. *BJOG.* 2005;112:1486-1491.

(15) Ness RB, Sibai BM. Shared and disparate components of the pathophysiologies of fetal growth restriction and preeclampsia. *Am J Obstet Gynecol.* 2006;195:40-49.

(16) Newstead J, von DP, Magee LA. Preeclampsia and future cardiovascular risk. *Expert Rev Cardiovasc Ther.* 2007;5:283-294.

(17) Mongraw-Chaffin ML, Cirillo PM, Cohn BA. Preeclampsia and cardiovascular disease death: prospective evidence from the child health and development studies cohort.

Hypertension. 2010;56:166-171.

(18) Ness RB, Hubel CA. Risk for coronary artery disease and morbid preeclampsia: a commentary. *Ann Epidemiol.* 2005;15:726-733.

(19) Harskamp RE, Zeeman GG. Preeclampsia: at risk for remote cardiovascular disease. *Am J Med Sci.* 2007;334:291-295.

(20) Ray JG, Vermeulen MJ, Schull MJ, Redelmeier DA. Cardiovascular health after maternal placental syndromes (CHAMPS): population-based retrospective cohort study. *Lancet.* 2005;366:1797-1803.

(21) Lazdam M, de la Horra A, Diesch J, Kenworthy Y, Davis E, Lewandowski AJ, Szmigielski C, Shore A, Mackillop L, Kharbanda R, Alp N, Redman C, Kelly B, Leeson P. Unique blood pressure characteristics in mother and offspring after early onset preeclampsia. *Hypertension.* 2012;60:1338-1345.

(22) Veerbeek JH, Hermes W, Breimer AY, van Rijn BB, Koenen SV, Mol BW, Franx A, de Groot CJ, Koster MP. Cardiovascular disease risk factors after early-onset preeclampsia, late-onset preeclampsia, and pregnancy-induced hypertension. *Hypertension.* 2015;65:600-606.

(23) Robbins CL, Hutchings Y, Dietz PM, Kuklina EV, Callaghan WM. History of preterm birth and subsequent cardiovascular disease: a systematic review. *Am J Obstet Gynecol.* 2014;210:285-297.

(24) Catov JM, Wu CS, Olsen J, Sutton-Tyrrell K, Li J, Nohr EA. Early or recurrent preterm birth and maternal cardiovascular disease risk. *Ann Epidemiol.* 2010;20:604-609.

(25) Romero R, Dey SK, Fisher SJ. Preterm labor: one syndrome, many causes. *Science.* 2014;345:760-765.

(26) Bonamy AK, Parikh NI, Cnattingius S, Ludvigsson JF, Ingelsson E. Birth characteristics and subsequent risks of maternal cardiovascular disease: effects of gestational age and fetal growth. *Circulation.* 2011;124:2839-2846.

(27) Oliver-Williams CT, Heydon EE, Smith GC, Wood AM. Miscarriage and future maternal cardiovascular disease: a systematic review and meta-analysis. *Heart.* 2013;99:1636-1644.

(28) Germain AM, Romanik MC, Guerra I, Solari S, Reyes MS, Johnson RJ, Price K, Karumanchi SA, Valdes G. Endothelial dysfunction: a link among preeclampsia, recurrent pregnancy loss, and future cardiovascular events? *Hypertension.* 2007;49:90-95.

(29) Sheiner E, Levy A, Katz M, Mazor M. Pregnancy outcome following recurrent spontaneous abortions. *Eur J Obstet Gynecol Reprod Biol.* 2005;118:61-65.

(30) Veerbeek JH, Smit JG, Koster MP, Post Uiterweer ED, van Rijn BB, Koenen SV, Franx A. Maternal cardiovascular risk profile after placental abruption. *Hypertension.* 2013;61:1297-1301.

(31) Shah BR, Retnakaran R, Booth GL. Increased risk of cardiovascular disease in young women following gestational diabetes mellitus. *Diabetes Care.* 2008;31:1668-1669.

(32) Metzger BE, Cho NH, Roston SM, Radvany R. Prepregnancy weight and antepartum insulin secretion predict glucose tolerance five years after gestational diabetes mellitus. *Diabetes Care.* 1993;16:1598-1605.

(33) Bellamy L, Casas JP, Hingorani AD, Williams D. Type 2 diabetes mellitus after gestational diabetes: a systematic review and meta-analysis. *Lancet.* 2009;373:1773-1779.

(34) Weissgerber TL, Mudd LM. Preeclampsia and diabetes. *Curr Diab Rep.* 2015;15:9.

(35) Briasoulis A, Bakris GL. Chronic kidney disease as a coronary artery disease risk equivalent. *Curr Cardiol Rep.* 2013;15:340.

(36) Dekker JM, Schouten EG. Number of pregnancies and risk of cardiovascular disease. *N Engl J Med.* 1993;329:1893-1894.

(37) Green A, Beral V, Moser K. Mortality in women in relation to their childbearing history. *BMJ.* 1988;297:391-395.

(38) Lawlor DA, Emberson JR, Ebrahim S, Whincup PH, Wannamethee SG, Walker M, Smith GD. Is the association between parity and coronary heart disease due to biological effects

of pregnancy or adverse lifestyle risk factors associated with child-rearing? Findings from the British Women's Heart and Health Study and the British Regional Heart Study. *Circulation.* 2003;107:1260-1264.

(39) Ness RB, Harris T, Cobb J, Flegal KM, Kelsey JL, Balanger A, Stunkard AJ, D'Agostino RB. Number of pregnancies and the subsequent risk of cardiovascular disease. *N Engl J Med.* 1993;328:1528-1533.

(40) Parikh NI, Cnattingius S, Dickman PW, Mittleman MA, Ludvigsson JF, Ingelsson E. Parity and risk of later-life maternal cardiovascular disease. *Am Heart J.* 2010;159:215-221.

(41) Colditz GA, Willett WC, Stampfer MJ, Rosner B, Speizer FE, Hennekens CH. A prospective study of age at menarche, parity, age at first birth, and coronary heart disease in women. *Am J Epidemiol.* 1987;126:861-870.

(42) Steenland K, Lally C, Thun M. Parity and coronary heart disease among women in the American Cancer Society CPS II population. *Epidemiology.* 1996;7:641-643.

(43) Ness RB, Cobb J, Harris T, D'Agostino RB. Does number of children increase the rate of coronary heart disease in men? *Epidemiology.* 1995;6:442-445.

(44) Halland F, deRoo L, Morken NH, Klungsoyr K, Wilcox AJ, Skjaerven R. [127-POS]: Educational level and maternal long-term risk of cardiovascular mortality by number of births. *Pregnancy Hypertens.* 2015;5:67.

(45) Parikh NI, Cnattingius S, Mittleman MA, Ludvigsson JF, Ingelsson E. Subfertility and risk of later life maternal cardiovascular disease. *Hum Reprod.* 2012;27:568-575.

(46) Rich-Edwards JW, Fraser A, Lawlor DA, Catov JM. Pregnancy characteristics and women's future cardiovascular health: an underused opportunity to improve women's health? *Epidemiol Rev.* 2014;36:57-70.

(47) Eskild A, Romundstad PR, Vatten LJ. Placental weight and birthweight: does the association differ between pregnancies with and without preeclampsia? *Am J Obstet*

Gynecol. 2009;201:595.

(48) Risnes KR, Romundstad PR, Nilsen TI, Eskild A, Vatten LJ. Placental weight relative to birth weight and long-term cardiovascular mortality: findings from a cohort of 31,307 men and women. *Am J Epidemiol.* 2009;170:622-631.

(49) Schwarz EB, Ray RM, Stuebe AM, Allison MA, Ness RB, Freiberg MS, Cauley JA. Duration of lactation and risk factors for maternal cardiovascular disease. *Obstet Gynecol.* 2009;113:974-982.

(50) Liu LX, Arany Z. Maternal cardiac metabolism in pregnancy. *Cardiovasc Res.* 2014;101:545-553.

(51) Broere-Brown ZA, Schalekamp-Timmermans S, Hofman A, Jaddoe V, Steegers E. Fetal sex dependency of maternal vascular adaptation to pregnancy: a prospective population-based cohort study. *BJOG.* 2015 (e-pub ahead of print). doi: 10.1111/1471-0528.13519

(52) Parikh NI, Lloyd-Jones DM, Ning H, Ouyang P, Polak JF, Lima JA, Bluemke D, Mittleman MA. Association of number of live births with left ventricular structure and function. The Multi-Ethnic Study of Atherosclerosis (MESA). *Am Heart J.* 2012;163:470-476.

(53) Martin U, Davies C, Hayavi S, Hartland A, Dunne F. Is normal pregnancy atherogenic? *Clin Sci (Lond).* 1999;96:421-425.

(54) Ness RB, Roberts JM. Heterogeneous causes constituting the single syndrome of preeclampsia: a hypothesis and its implications. *Am J Obstet Gynecol.* 1996;175:1365-1370.

(55) Buurma AJ, Turner RJ, Driessen JH, Mooyaart AL, Schoones JW, Bruijn JA, Bloemenkamp KW, Dekkers OM, Baelde HJ. Genetic variants in pre-eclampsia: a meta-analysis. *Hum Reprod Update.* 2013;19:289-303.

(56) Johnson MP, Brennecke SP, East CE, Dyer TD, Roten LT, Proffitt JM, Melton PE, Fenstad MH, Aalto-Viljakainen T, Makikallio K, Heinonen S, Kajantie E, Kere J, Laivuori

H, Austgulen R, Blangero J, Moses EK. Genetic dissection of the pre-eclampsia susceptibility locus on chromosome 2q22 reveals shared novel risk factors for cardiovascular disease. *Mol Hum Reprod.* 2013;19:423-437.

(57) Redman CW, Sacks GP, Sargent IL. Preeclampsia: an excessive maternal inflammatory response to pregnancy. *Am J Obstet Gynecol.* 1999;180:499-506.

(58) Ross R. Atherosclerosis is an inflammatory disease. *Am Heart J.* 1999;138:S419-S420.

(59) Ross R. Atherosclerosis--an inflammatory disease. *N Engl J Med.* 1999;340:115-126.

(60) Rosing U, Samsioe G, Olund A, Johansson B, Kallner A. Serum levels of apolipoprotein A-I, A-II and HDL-cholesterol in second half of normal pregnancy and in pregnancy complicated by pre-eclampsia. *Horm Metab Res.* 1989;21:376-382.

(61) Hubel CA, Lyall F, Weissfeld L, Gandley RE, Roberts JM. Small low-density lipoproteins and vascular cell adhesion molecule-1 are increased in association with hyperlipidemia in preeclampsia. *Metabolism.* 1998;47:1281-1288.

(62) Sattar N, Bendomir A, Berry C, Shepherd J, Greer IA, Packard CJ. Lipoprotein subfraction concentrations in preeclampsia: pathogenic parallels to atherosclerosis. *Obstet Gynecol.* 1997;89:403-408.

(63) Wetzka B, Winkler K, Kinner M, Friedrich I, Marz W, Zahradnik HP. Altered lipid metabolism in preeclampsia and HELLP syndrome: links to enhanced platelet reactivity and fetal growth. *Semin Thromb Hemost.* 1999;25:455-462.

(64) Ware-Jauregui S, Sanchez SE, Zhang C, Laraburre G, King IB, Williams MA. Plasma lipid concentrations in pre-eclamptic and normotensive Peruvian women. *Int J Gynaecol Obstet.* 1999;67:147-155.

(65) Staff AC, Dechend R, Pijnenborg R. Learning from the placenta: acute atherosis and vascular remodeling in preeclampsia-novel aspects for atherosclerosis and future cardiovascular health. *Hypertension.* 2010;56:1026-1034.

(66) Barden A. Pre-eclampsia: contribution of maternal constitutional factors and the

consequences for cardiovascular health. *Clin Exp Pharmacol Physiol.* 2006;33:826-830.

(67) Wikstrom AK, Stephansson O, Cnattingius S. Tobacco use during pregnancy and preeclampsia risk: effects of cigarette smoking and snuff. *Hypertension.* 2010;55:1254-1259.

(68) Cudmore M, Ahmad S, Al-Ani B, Fujisawa T, Coxall H, Chudasama K, Devey LR, Wigmore SJ, Abbas A, Hewett PW, Ahmed A. Negative regulation of soluble Flt-1 and soluble endoglin release by heme oxygenase-1. *Circulation.* 2007;115:1789-1797.

(69) Jeyabalan A, Powers RW, Durica AR, Harger GF, Roberts JM, Ness RB. Cigarette smoke exposure and angiogenic factors in pregnancy and preeclampsia. *Am J Hypertens.* 2008;21:943-947.

(70) Magnussen EB, Vatten LJ, Lund-Nilsen TI, Salvesen KA, Davey SG, Romundstad PR. Prepregnancy cardiovascular risk factors as predictors of pre-eclampsia: population based cohort study. *BMJ.* 2007;335:978.

(71) Magnussen EB, Vatten LJ, Smith GD, Romundstad PR. Hypertensive disorders in pregnancy and subsequently measured cardiovascular risk factors. *Obstet Gynecol.* 2009;114:961-970.

(72) Romundstad PR, Magnussen EB, Smith GD, Vatten LJ. Hypertension in pregnancy and later cardiovascular risk: common antecedents? *Circulation.* 2010;122:579-584.

(73) Staff AC, Dechend R, Redman CWG. Review: Preeclampsia, acute atherosis of the spiral arteries and future cardiovascular disease: two new hypotheses. *Placenta.* 2012;34 Suppl:S73-8.

(74) Staff AC, Dechend R, Redman CW. Review: Preeclampsia, acute atherosis of the spiral arteries and future cardiovascular disease: two new hypotheses. *Placenta.* 2013;34 Suppl:S73-S78.

(75) Melchiorre K, Sharma R, Thilaganathan B. Cardiovascular implications in preeclampsia: an overview. *Circulation.* 2014;130:703-714.

(76) Staff AC, Benton SJ, von DP, Roberts JM, Taylor RN, Powers RW, Charnock-Jones DS, Redman CW. Redefining Preeclampsia Using Placenta-Derived Biomarkers. *Hypertension.* 2013;61:932-942.

(77) Kvehaugen AS, Dechend R, Ramstad HB, Troisi R, Fugelseth D, Staff AC. Endothelial function and circulating biomarkers are disturbed in women and children after preeclampsia. *Hypertension.* 2011;58:63-69.

(78) Wolf M, Hubel CA, Lam C, Sampson M, Ecker JL, Ness RB, Rajakumar A, Daftary A, Shakir AS, Seely EW, Roberts JM, Sukhatme VP, Karumanchi SA, Thadhani R. Preeclampsia and future cardiovascular disease: potential role of altered angiogenesis and insulin resistance. *J Clin Endocrinol Metab.* 2004;89:6239-6243.

(79) Noori M, Donald AE, Angelakopoulou A, Hingorani AD, Williams DJ. Prospective study of placental angiogenic factors and maternal vascular function before and after preeclampsia and gestational hypertension. *Circulation.* 2010;122:478-487.

(80) Saxena AR, Karumanchi SA, Brown NJ, Royle CM, McElrath TF, Seely EW. Increased sensitivity to angiotensin II is present postpartum in women with a history of hypertensive pregnancy. *Hypertension.* 2010;55:1239-1245.

(81) Hubel CA, Wallukat G, Wolf M, Herse F, Rajakumar A, Roberts JM, Markovic N, Thadhani R, Luft FC, Dechend R. Agonistic angiotensin II type 1 receptor autoantibodies in postpartum women with a history of preeclampsia. *Hypertension.* 2007;49:612-617.

(82) Bytautiene E, Bulayeva N, Bhat G, Li L, Rosenblatt KP, Saade GR. Long-term alterations in maternal plasma proteome after sFlt1-induced preeclampsia in mice. *Am J Obstet Gynecol.* 2013;208:388.

(83) Lin C, Rajakumar A, Plymire DA, Verma V, Markovic N, Hubel CA. Maternal Endothelial Progenitor Colony-Forming Units With Macrophage Characteristics Are Reduced in Preeclampsia. *Am J Hypertens.* 2009;22:1014-1019.

(84) Autiero M, Waltenberger J, Communi D, Kranz A, Moons L, Lambrechts D, Kroll J,

Plaisance S, De Mol M, Bono F, Kliche S, Fellbrich G, Ballmer-Hofer K, Maglione D, Mayr-Beyrle U, Dewerchin M, Dombrowski S, Stanimirovic D, Van Hummelen P, Dehio C, Hicklin DJ, Persico G, Herbert JM, Communi D, Shibuya M, Collen D, Conway EM, Carmeliet P. Role of PlGF in the intra- and intermolecular cross talk between the VEGF receptors Flt1 and Flk1. *Nat Med.* 2003;9:936-943.

(85) Li B, Sharpe EE, Maupin AB, Teleron AA, Pyle AL, Carmeliet P, Young PP. VEGF and PlGF promote adult vasculogenesis by enhancing EPC recruitment and vessel formation at the site of tumor neovascularization. *FASEB J.* 2006;20:1495-1497.

(86) Melchiorre K, Sutherland GR, Liberati M, Thilaganathan B. Preeclampsia is associated with persistent postpartum cardiovascular impairment. *Hypertension.* 2011;58:709-715.

(87) Kane GC, Karon BL, Mahoney DW, Redfield MM, Roger VL, Burnett JC, Jr., Jacobsen SJ, Rodeheffer RJ. Progression of left ventricular diastolic dysfunction and risk of heart failure. *JAMA.* 2011;306:856-863.

(88) Redfield MM, Jacobsen SJ, Burnett JC, Jr., Mahoney DW, Bailey KR, Rodeheffer RJ. Burden of systolic and diastolic ventricular dysfunction in the community: appreciating the scope of the heart failure epidemic. *JAMA.* 2003;289:194-202.

(89) Roberts JM, Catov JM. Pregnancy is a screening test for later life cardiovascular disease: now what? Research recommendations. *Womens Health Issues.* 2012;22:e123-e128.

(90) Pinsky JL, Branch LG, Jette AM, Haynes SG, Feinleib M, Cornoni-Huntley JC, Bailey KR. Framingham Disability Study: relationship of disability to cardiovascular risk factors among persons free of diagnosed cardiovascular disease. *Am J Epidemiol.* 1985;122:644-656.

(91) Mosca L, Benjamin EJ, Berra K, Bezanson JL, Dolor RJ, Lloyd-Jones DM, Newby LK, Pina IL, Roger VL, Shaw LJ, Zhao D, Beckie TM, Bushnell C, D'Armiento J, Kris-Etherton PM, Fang J, Ganiats TG, Gomes AS, Gracia CR, Haan CK, Jackson EA, Judelson DR, Kelepouris E, Lavie CJ, Moore A, Nussmeier NA, Ofili E, Oparil S, Ouyang

P, Pinn VW, Sherif K, Smith SC, Jr., Sopko G, Chandra-Strobos N, Urbina EM, Vaccarino V, Wenger NK. Effectiveness-based guidelines for the prevention of cardiovascular disease in women--2011 update: a guideline from the American Heart Association. *J Am Coll Cardiol.* 2011;57:1404-1423.

(92) Lenderink T, Heeschen C, Fichtlscherer S, Dimmeler S, Hamm CW, Zeiher AM, Simoons ML, Boersma E. Elevated placental growth factor levels are associated with adverse outcomes at four-year follow-up in patients with acute coronary syndromes. *J Am Coll Cardiol.* 2006;47:307-311.

(93) Hypertension in pregnancy. Report of the American College of Obstetricians and Gynecologists' Task Force on Hypertension in Pregnancy. *Obstet Gynecol.* 2013;122:1122-1131.

(94) National Institute for Health and Clinical Excellence. *Hypertension in pregnancy: the management of hypertensive disorders during pregnancy (clinical guideline 107).2010.* 2013.

(95) Myatt L, Redman CW, Staff AC, Hansson S, Wilson ML, Laivuori H, Poston L, Roberts JM. Strategy for Standardization of Preeclampsia Research Study Design. *Hypertension.* 2014;63:1293-301.

(96) Burton GJ, Sebire NJ, Myatt L, Tannetta D, Wang YL, Sadovsky Y, Staff AC, Redman CW. Optimising sample collection for placental research. *Placenta.* 2014;35:9-22.

(97) Burke O, Benton S, Szafranski P, von DP, Buhimschi C, Cetin I, Chapell L, Figueras F, Galindo A, Herraiz I, Holzman C, Hubel C, Knudsen U, Kronborg C, Laivuori H, McElrath T, Moertl M, Meyers J, Ness RB, Oliviera L, Olson G, Poston L, Ris-Stalpers C, Roberts JM, Schistermann E, Steegers E, Stepan H, Lapaire O, Schlemback D, Timmermans S, Tsatsaris V, van der Post JA, Verlohren S, Villa PM, Williams D, Zeisler H, Zhang C, Redman C, Staff AC. [94-OR]: Extending the scope of individual patient data meta-analyses: Merging algorithms for biomarker measurements from heterogeneous

laboratory platforms. The CoLAB Preeclampsia angiogenic factor study. *Pregnancy Hypertens.* 2015;5:50-51.

(98)   Jaddoe VW, Bakker R, van Duijn CM, van der Heijden AJ, Lindemans J, Mackenbach JP, Moll HA, Steegers EA, Tiemeier H, Uitterlinden AG, Verhulst FC, Hofman A. The Generation R Study Biobank: a resource for epidemiological studies in children and their parents. *Eur J Epidemiol.* 2007;22:917-923.

(99)   Kruithof CJ, Kooijman MN, van Duijn CM, Franco OH, de Jongste JC, Klaver CC, Mackenbach JP, Moll HA, Raat H, Rings EH, Rivadeneira F, Steegers EA, Tiemeier H, Uitterlinden AG, Verhulst FC, Wolvius EB, Hofman A, Jaddoe VW. The Generation R Study: Biobank update 2015. *Eur J Epidemiol.* 2014;29:911-927.

(100) Gishti O, Jaddoe VW, Felix JF, Reiss I, Hofman A, Ikram MK, Steegers EA, Gaillard R. Influence of Maternal Angiogenic Factors During Pregnancy on Microvascular Structure in School-Age Children. *Hypertension.* 2015;65:722-8.

(101) Ronningen KS, Paltiel L, Meltzer HM, Nordhagen R, Lie KK, Hovengen R, Haugen M, Nystad W, Magnus P, Hoppin JA. The biobank of the Norwegian Mother and Child Cohort Study: a resource for the next 100 years. *Eur J Epidemiol.* 2006;21:619-625.

(102) Magnus P, Irgens LM, Haug K, Nystad W, Skjaerven R, Stoltenberg C. Cohort profile: the Norwegian Mother and Child Cohort Study (MoBa). *Int J Epidemiol.* 2006;35:1146-1150.

(103) Harrison S, Petrovic G, Chevassut A, Brook L, Higgins N, Kenworthy Y, Selwood M, Snelgar T, Arnold L, Boardman H, Heneghan C, Leeson P, Redman C, Granne I. Oxfordshire Women and Their Children's Health (OxWATCH): Protocol for a prospective cohort feasibility study. Submitted to BMJ Open July 2015.

(104) Ala-Korpela M. Critical evaluation of 1H NMR metabonomics of serum as a methodology for disease risk assessment and diagnostics. *Clin Chem Lab Med.* 2008;46:27-42.

(105) Petersen SE, Matthews PM, Bamberg F, Bluemke DA, Francis JM, Friedrich MG, Leeson P, Nagel E, Plein S, Rademakers FE, Young AA, Garratt S, Peakman T, Sellors J, Collins

R, Neubauer S. Imaging in population science: cardiovascular magnetic resonance in 100,000 participants of UK Biobank - rationale, challenges and approaches. *J Cardiovasc Magn Reson.* 2013;15:46.

(106) Global, regional, and national age-sex specific all-cause and cause-specific mortality for 240 causes of death, 1990-2013: a systematic analysis for the Global Burden of Disease Study 2013. *Lancet.* 2015;385:117-171.

(107) Seely EW, Rich-Edwards J, Lui J, Nicklas JM, Saxena A, Tsigas E, Levkoff SE. Risk of future cardiovascular disease in women with prior preeclampsia: a focus group study. *BMC Pregnancy Childbirth.* 2013;13:240.

(108) Young B, Hacker MR, Rana S. Physicians' knowledge of future vascular disease in women with preeclampsia. *Hypertens Pregnancy.* 2012;31:50-58.

(109) Visintin C, Mugglestone MA, Almerie MQ, Nherera LM, James D, Walkinshaw S. Management of hypertensive disorders during pregnancy: summary of NICE guidance. *BMJ.* 2010;341:c2207.

Novelty and significance*:*

1) *What is new:*
- A template is specified for harmonizing methodologies of follow-up studies after pregnancies, complicated by placental dysfunction or gestational diabetes, which aim to determine the mechanisms, and quantify the risks, of future cardiovascular disease.

2) *What is relevant:*
- The template will facilitate aggregation of data and biosamples between studies, so enhancing their power to increase understanding of all aspects of the evolution of cardiovascular risks after abnormal pregnancies.

3) *Summary:*
- More powerful long-term studies, of the relevant early biomarkers, will aid targeting women at highest risk of cardiovascular disease for preventive interventions, decades before a lethal event.

	AHA(1)	ACOG (2)	ADA (3)	NICE (4, 5)	SOMANZ (6)	ADIPS (7)
Preeclampsia, FGR, GDM and Premature Delivery	**Assessments:** BP, Lipids, Fasting blood glucose, BMI **Lifestyle advice:** BMI<25kg/m^2 Healthy diet Physical activity No smoking					
Hypertensive Disorder of Pregnancy		**Assessments** (yearly if preterm PE or recurrent PE)**:** BP, Lipids, Fasting blood glucose, BMI **Lifestyle advice:** Maintain maternal weight Physical activity No smoking		**Information to women:** Increased risk of gestational hypertension/PE in future pregnancy Increased risk of hypertension and its complications later in life **Lifestyle advice:** Maintain maternal weight (BMI 18.5-24.9 kg/m^2). Healthy diet	**Assessments:** BP (yearly) Lipids (every 5 years) Glucose (every 5 years) **Lifestyle advice:** Maintain maternal weight Healthy diet Physical activity No smoking	
Gestational Diabetes Mellitus		**Assessments:** OGTT (6 weeks postpartum)	**Assessments:** Screen for diabetes (6-12 weeks postpartum and every 1-3 years)	**Information:** GDM risk next pregnancy Symptoms of hyperglycemia **Assessments:** Fasting plasma glucose before hospital discharge Fasting plasma glucose (6-13 weeks postpartum) Test for diabetes when planning next pregnancy **Lifestyle advice:** Maintain maternal weight Healthy diet Physical activity		**Assessments:** OGTT (6-12 weeks postpartum) Fasting plasma glucose/HbA1C (at least every 1-2 years)

Supplemental Table 1 (S1): Examples of current guidelines on clinical follow-up for future **cardiovascular** disease (CVD) after a pregnancy outcome associated with increased CVD risk

BP: blood pressure; FGR: fetal growth restriction; GDM: gestational diabetes mellitus; OGTT: Oral glucose tolerance test; PE: preeclampsia

1.   Mosca L, Benjamin EJ, Berra K, Bezanson JL, Dolor RJ, Lloyd-Jones DM, et al. Effectiveness-based guidelines for the prevention of cardiovascular disease in women--2011 update: a guideline from the American Heart Association. J Am Coll Cardiol. 2011;57(12):1404-23.
2.   Hypertension in pregnancy. Report of the American College of Obstetricians and Gynecologists' Task Force on Hypertension in Pregnancy. Obstet Gynecol. 2013;122(5):1122-31.
3.   Management of diabetes in pregnancy. Diabetes Care. 2015;38 Suppl:S77-9.
4.   Visintin C, Mugglestone MA, Almerie MQ, Nherera LM, James D, Walkinshaw S. Management of hypertensive disorders during pregnancy: summary of NICE guidance. BMJ. 2010;341:c2207.
5.   National Institute for Health and Clinical Excellence. Diabetes in pregnancy: management of diabetes and its complications from preconception to the postnatal period. 16 Postnatal care. http://www.nice.org.uk/guidance/ng3/chapter/1-recommendations2015.
6.   The SOMANZ Guideline for the Management of Hypertensive Disorders of Pregnancy, (2014).
7.   Nankervis A, MacIntyre HD, Moses R, Ross GP, Callaway L, Porter C, et al. ADIPS Consensus Guidelines for the Testing and Diagnosis of Hyperglycaemia in Pregnancy in Australia and New Zealand. 2014.

TABLE 1: Harmonization of research studies for future CVD follow-up in low risk young women; suggested "minimal" (green marked) and *"extended" (Itallic)* dataset

VISIT	Clinical information	Biological samples for research biobanking	CV risk phenotyping
Pregestational	**Family history of CVD:** CVD/CVD death in 1st degree relative, type of CVD, age at time of diagnosis or death **Basics (Physical, anthropological and ethnographic data): Age, height and weight (BMI) and waist/hip ratio** **Smoking history** (never, irregularly, regularly use, current use): Cigarette/Cigar or snuff or chews tobacco/nicotine **Medical history :** Hypertension, cardiac disease, stroke, renal disease, pregestational DM (type and treatment), collagen vascular disease, systemic lupus erythematosus, obstructive sleep apnea **Obstetric history** (gravidity, parity: indicate numbers and gestational age at deliveries): Miscarriage, stillbirth, abortions (induced/spontaneous), Pregnancy induced hypertension, preeclampsia, eclampsia, HELLP, Small for gestational age/Fetal growth restriction, Gestational DM (treatment type), preterm delivery (<37 weeks), neonatal death, placental weights Self-described ethnicity (White, Black, Asian, Hispanic, unknown, or other [mixed]) *Years of schooling/other socioeconomic indicator* *Maternal/paternal (and grandparent) country of birth* *Physical activity (IPAQ) and Diet questionnaires* *Breast feeding history (duration and after how many pregnancies?)*	*Blood (plasma and serum) and urine sampling*	**BP (blood pressure),** measured and reported according to accepted guidelines **Blood screen** for: Dyslipidemia (total cholesterol, LDL-cholesterol, HDL-cholesterol) and Diabetes Mellitus (fasting blood glucose or HbA1c) **Urine screen**: protein (and hematuria/ glucosuria) *Cardiovascular phenotyping 1: Macrovasculature Function (Endothelial function and Arterial stiffness) and Structure (Carotid imaging)* *Cardiovascular phenotyping 2 : Microvasculature (rarefaction)* *Cardiovascular phenotyping 3: Echocardiography* *Oral glucose tolerance test (OGT)/ HOMA score*
Pregnancies (all trimesters preferrably)	**Updated Family history of CVD, basics, smoking, medical/ obstetric history (as in pregestational visit above)** Pregnancy clinical information, including maternal/fetal outcome and placenta variables (ref Myatt al, Hypertension 2014) *US registrations from pregnancy: Uteroplacental Doppler blood flow findings and fetal growth measurements*	*Longitudi nal blood (plasma and serum) and urine sampling, according to Myatt et al, Hypertension 2014* *Placental sampling, according to Burton et al, Placenta 2014*	**BP,** measured and reported according to accepted guidelines (see S1 references for pregnancy BP) **Urine screen**: protein (and hematuria/glucosuria and UTI screen). Albumin/creatinine ratio (longitudinal, until positive diagnosis of proteniuria/preeclampsia) **OGT/** HOMA score *Cardiovascular phenotyping:1-3 (as above)*
Postpartum (6-12 weeks, 6 months and 1 year after index pregnancy, then every 5th year)	**Update of family history of CVD, basics, smoking, medical / obstetric history (as in pregestational visit above)** *Physical activity (IPAQ) and Diet questionnaires* *Breast feeding history (duration)*	*Blood (plasma and serum) and urine sampling*	**BP,** measured and reported according to accepted guidelines **Urine screen**: protein (and hematuria/ glucosuria) **Blood screen** for: Dyslipidemia (total cholesterol, LDL-cholesterol, HDL-cholesterol) and Diabetes Mellitus (fasting blood glucose or HbA1c) *Oral glucose tolerance test (3-6 months postpartum after GDM is recommended clinically)* *Cardiovascular phenotyping:1-3 (as above)*